A HISTOLOGICAL EVALUATION OF LIVERS FROM FISH RESIDENT TO SELECTED REACHES OF THE ILLINOIS RIVER

FINAL REPORT

SUBMITTED TO:
Ms. Jody Millar
Assistant Field Supervisor
Ash Contaminants
US Fish and Wildlife Service
1830 Second Avenue
Rock Island, Illinois 61201

SUBMITTED BY:

Dr. Eric B. May
Senior Associate
MB Aquapath
Box 246 A5D
Queenstown, Maryland 21658

INTRODUCTION

The following report is based on fifty livers taken from a variety of fish species resident to the Illinois River. Of the fifty fish taken and necropsied, livers designated as IL 43 through 50 were from frozen samples, however were unsuitable for the purpose of this report.

The basis of histological examination of the livers was to see if there are any changes which would reflect altered habitats and thereby cause disease (infectious and non-infectious) among the resident fish, or if lesions were present which would reflect conditions that may pose a significant threat to human health. Since many toxins are found to exert effects at the organ and tissue lever before evidence of clinical disease is seen, this technology is useful as an indicator in most circumstances.

Historically, histological evaluations have been used primarily to detect tumors in major organs. In this case it has been used to determine if both tumors or sublethal (subclinical) effects are present. The effects would be likely due to habitat conditions or contamination from anthropogenic sources.

METHODOLOGY

Tissues were received by this laboratory as Bouin's fixed tissues in glass containers. The tissues were examined, trimmed and placed in cassettes for processing for paraffin embedment following standard protocol. The processed tissue was then sectioned at 6.0 microns, adhered to clean glass slides, stained using hematoxylin and eosin, and covered using permount and clean coverglass. All tissues were then examined for lesions, and these were reported using standard veterinary terminology, and coded using a modified coding system developed at this laboratory (SEE INSERTS 1 AND 2).

INSERT 1: Explanation of Codes for Organ, Sub-organ Location and Lesions

Organ and Sub-organ location

GS, LI = Gastrointestinal System, Liver
GS, PA = Gastrointestinal System, Pancreas
HP = Hepatocytes
BD = Bile Ducts

Lesions

GS, LI
AR, FX = An artifact created by fixation which alters the tissue
AU = Autolysis or rotting of liver
BD, DY = Dystrophic or malformation of bile ducts
BD, PA, CE = Parasitic infestation of cestodes
BD, PA, PR = Parasitic infestation of protozoa
BD, PA, TR = Parasitic infestation of trematodes
BD, PC, CH = Pericholangitis or inflammation of the tissue surrounding bile ducts, in this case infiltrates of mononuclear cells (chronic)
BD, PC, GR, VE = Pericholangitis or inflammation of the tissue surrounding bile ducts, in this case granulomatous (long chronic) of parasitic origin
BD, RC = Presence of rodlet cells in the bile ducts
BD, TU, CC, MC = Tumor of the bile ducts, characterized as a cholangiocystadenoma occurring in multiple locations (multicentric)
HP, VC, FC = Vacuolar change of fatty infiltrate origin in individual hepatocytes
HP, VC, HY = Vacuolar change of hydropic origin in hepatocytes
HT, CH = Hepatitis characterized by infiltrates of mononuclear cells (chronic) in interstitial tissue
HT, GR, VE = Hepatitis characterized by granuloma of parasitic origin
HT, NE = Hepatitis characterized by necrosis of hepatocytes without evidence of inflammatory cell infiltrates
MA, IN, SI and NO = Increase in macrophage aggregates both in size of individual aggregates, and number of aggregates seen
NS = No significant lesions
PA, TR = Parasitic infiltrates of trematodes into liver interstitial tissue

Severity
mi = mild
mo = moderate
se = severe

INSERT 2: Explanation of Species Codes for Fish Examined

BG = Bluegill
CA = Carp
CC = Channel Catfish
FD = Freshwater Drum
GF = Goldfish
GR = Golden Redhorse
MI = Mixed species from frozen liver
QC = Quillback Carp
SB = Smallmouth Bass
SR = Shorthead Redhorse
YB = Yellow Bullhead
-R = Redhorse of Unknown Type

RESULTS

Lesions observed are listed as Insert 1. All are under the GS:LI:BD or HP as location codes, or HT, AU, or PA as stromal or condition codes. Bile duct lesions (BD) were seen as mild to moderate pericholangitis (PC) which usually was focal and not considered significant. Parasitic infestation of bile ducts was frequent which is normal. In fact, the absence of parasites either in the liver or bile ducts would have been considered abnormal. Of greater concern was the detection of bile ductule dystrophy and a tumor (cholangiocystadenoma). The dystrophy is at present unresolved with hyperplasia and/or metaplasia being considered as the final diagnoses. In either case the lesion represents a pre-neoplastic condition which could at some point become a neoplasm, had the fish lived. The "tumor" or neoplasm has been designated as a cholangiocystadenoma. Both the pre-neoplastic and neoplastic lesions were seen only in carp livers, and only in carp taken at the I-55 Bridge (see Table 1) or at the Brandon Road Tailwaters (see Table 2). Of seven carp from the I-55 collection two exhibited the pre-neoplastic lesions (BD,DY) and two were found to have the cholangiocystadenoma (BD,TU,CC,MC). The cholangiocystadenomas are interesting, in that, instead of being a single focus of origin they are multicentric (MC) or seen a multiple locations in the liver. What ever is inducing these lesions must for some individuals exert a strong effect. In the carp from the Brandon Road Tailwaters two of four were seen to exhibit the cholangiocystadenoma (see Table 2) and one of these also had evidence of bile ductule dystrophy. In the two collections rodlet cells were seen integrated with the lining cells of the bile ducts (BD,RC). In one case where dystrophy was the most pronounced the rodlet cells appeared involved with the dystrophic process.

Lesions of the liver parenchyma (liver cells) were classed as vacuolar change of either hydropic (water) or fatty infiltrates (HP,VC,FC or HY). The hydropic change is a normal finding where individuals are under a mild level of "stress". This is a nonspecific change and can not be considered significant. It was however most prominent in the I-55 collection (see Table 1) as compared to other collections (see Tables 2 through 5). Inflammation of chronic (HT,CH), parasitic (HT,GR,VE) or acute (HT,NE) nature were seen at low levels of incidence at most locations. These were focal lesions and are not considered significant.

Increases in the size of the melanomacrophage aggregates (MA,IN,SI,NO) was seen in many cases. This lesion, characterized by accumulations of macrophages, is a nonspecific change and indicates past or current response to some "stressor". In some cases no lesions were seen (NS) and in one case stromal (interstitial) trematodes were seen (see Table 4).

TABLE 1: Lesions present in fish livers collected 7-1-91 at I-55 Bridge over Illinois River

FISH #	AR FX	AU	BD DY	BD PA CE	BD PA PR	BD PA TR	BD PC CH	BD PC GR VE	BD RC	BD TU CC MC	HP VC FC	HP VC HY	HT CH	HT GR VE	HT NE	MA IN SI NO	NS	PA TR
1 CA								mi				mi	mi					
2 CA			mo		mo								mi		mo	se		
3 CA							mi		mo	mc			mo					
4 CA			mo						mo			mi						
5 CA							mi			mc		mo						
6 CA												se						
7 GF																	x	
8 GF																	x	
9 CC	mo																x	
10 SR							mi											
11 GR	mo																x	
12 -R											mo							
13 BG											mi	mi						
14 FD					mi							mo						
15 GF												mo						
16 CA												mo						
N =16	2		2		2		3	1	2	2	2	8	3		1	1	4	

TABLE 2: Lesions present in fish livers collected 7-2-91 from the Brandon Road Tailwaters

	AR FX	AU	BD DY	BD PA CE	BD PA PR	BD PA TR	BD PC CH	BD PC GR VE	BD RC	BD TU CC MC	HP VC FC	HP VC HY	HT CH	HT GR VE	HT NE	MA IN SI NO	NS	PA TR
FISH #																		
17 CA							mi			mc		mi					x	
18 CA																se		
19 CA			mo				mi		mo	mc		mi						
20 CA												mi						
N = 4			1				2		1	2		3				1	1	

TABLE 3: Lesions present in fish livers collected 7-2-91 from Sugar Island

	AR FX	AU	BD DY	BD PA CE	BD PA PR	BD PA TR	BD PC CH	BD PC GR VE	BD RC	BD TU CC MC	HP VC FC	HP VC HY	HT CH	HT GR VE	HT NE	MA IN SI NO	NS	PA TR
FISH #																		
21	CA											mi						
22	CA											mi						
23	YB													mi				
30	YB																x	
31	YB																x	
N = 5												2		1			2	

TABLE 4: Lesions present in fish livers collected 7-2-91 from Dresden Tailwaters

	AR FX	AU	BD DY	BD PA CE	BD PA PR	BD PA TR	BD PC CH	BD PC GR VE	BD RC	BD TU CC MC	HP VC FC	HP VC HY	HT CH	HT GR VE	HT NE	MA IN SI NO	NS	PA TR
FISH #																		
24 CC														se				
25 CC																	x	
26 CC												mi						
27 SB				mi										mo				
28 SB														mo				mo
29 FD					mi											mo		
32 FD						mo										se		
33 FD							se							se		se		
N = 8				1	1	1	1					1		4		3	1	1

TABLE 5: Lesions present in fish livers collected 7-3-91 at Marseilles Tailwaters

	ARFX	AU	BDDY	BDPACE	BDPAPR	BDPATR	BDPCCH	BDPCGRVE	BDRC	BDTUCCMC	HPVCFC	HPVCHY	HTCH	HTGRVE	HTNE	MAINSINO	NS	PATR
34 CA							mi											
35 CA																mi		
36 FD																	x	
37 FD														mo		mo		
38 FD																	x	
39 FD												mi						
40 SR											mi							
41 CA							mo					mi						
42 QC							mi											
43-50 MI		se																
N = 17		8					3				2	2		1		2	2	

DISCUSSION

In general the lesions seen in the livers were not severe as other livers examined by this author taken from fish resident to altered habitats. On the other hand, this is the probably the first report of cholangiocystadenomas in carp, and the first of bile ductule dystrophy (see Tables 1 and 2). Whether these lesions are related to the appearance of rodlet cells, or due to a contaminant is not known. Arguments for both sides of the question exist. Rodlet cells are considered to be parasites which through evolution have become completely integrated with the host. Carp are particularly susceptible. Some on the other hand, argue that the rodlet cells are normal fish cells whose function remains unknown. This author after twenty years takes no side in the issue as the correct studies to elucidate the answer have yet to be performed.

The conclusions that can be reached are:

1. The collections from I-55 Bridge and Brandon Road Tailwaters yielded higher than expected rates of dystrophic bile ducts and tumors classified as cholangiocystadenomas.

2. Both lesions are pre-neoplastic or neoplastic in nature.

3. No other location yielded these results.

Since the collections involved a variety of species, statistical comparisons can not be made. If future investigations are needed, I recommend the following:

1. Two species (carp and brown bullheads) be taken at each site being investigated. Carp because of the existing lesions, bullheads as they are good indicators (rapid histological responses).

2. Full necropsies be performed, taking the brain, kidney (head and caudal), gill, liver, spleen, stomach, intestines, muscle, bone and skin.

3. Samples of water and sediments be taken for chemical analysis. Samples should be collected at the center of the reach where fish are taken.

What are the human implications, we do not know. However, to answer this in part, samples of flesh should be taken and analyzed for significant organic compounds considered to exhibit a carcinogenic potential.

Dr. Eric B. May, Senior Associate — 9-24-91 Date